AD_____

Award Number: W81XWH-18-1-0094

TITLE: A Fetus-Targeted Antibody Therapy to Prevent Zika Virus Infection During Pregnancy

PRINCIPAL INVESTIGATOR: Diogo Magnani

CONTRACTING ORGANIZATION: University of Massachusetts Worcester, 01655-0002

REPORT DATE: May 2019

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

√ Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
May 2019	**Annual**	*1 May 2018 - 30 Apr 2019*

4. TITLE AND SUBTITLE
A Fetus-Targeted Antibody Therapy to Prevent Zika Virus Infection During Pregnancy

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0094

5c. PROGRAM ELEMENT NUMBER

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

6. AUTHOR(S)

Diogo Magnani

E-Mail: Diogo.Magnani@umassmed.edu

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Massachusetts, Worcester, 01655-0002

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

14. ABSTRACT

Interventions are urgently needed to prevent the severe fetal complications of Zika virus infection. In the US, one in ten pregnant women infected with Zika virus delivered babies with severe birth defects, including small head size (microcephaly) and other neurological problems. Once the fetus is infected, there is nothing we can do to stop viral replication with current technologies. An approach considered safe for the fetus is to administer antibodies that can neutralize Zika virus. The problem, however, is that these effective antivirals, cannot reach the fetal tissues early in the pregnancy in sufficient amounts to be effective. Here, we are proposing to engineer antibody molecules so that they will cross the placenta effectively and reach the fetus. We will test our approach in rhesus monkeys, the best animal model of Zika virus infections during human pregnancy, by administering our 'fetus-targeted' antibody to pregnant macaques and challenging with Zika virus. If the antibodies can indeed prevent fetal infection on macaques, this proposal might lead to one of the first effective therapies for Zika virus infection.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:

a. REPORT
Unclassified

	b. ABSTRACT	**c. THIS PAGE**	**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
	Unclassified	Unclassified	Unclassified		USAMRMC
					19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

1. **INTRODUCTION:**

Our ultimate objective is to prevent fetal Zika virus (ZIKV) infection. We are testing a novel approach for preventing fetal ZIKV replication, by engineering monoclonal antibody (mAb) delivery to the fetus. We have two major aims, to **define mAb mutations that result in enhanced transplacental transport** and to test if a **fetus-targeted mAb can prevent congenital ZIKV infection.** The proposed experiments are straightforward: we are engineering a ZIKV-neutralizing antibody with neonatal Fc receptor (FcRn)-enhancing mutations and testing *in vitro*. The ability of this antibody to cross the placenta and prevent ZIKV infection will be tested *in vivo* in the last 12 months of the award. This award was transferred to the University of Massachusetts Medical School (UMass). This modification in project site resulted in a delayed start date. Therefore, we are now reporting the early progress obtained in the initial six months of the grant.

2. **KEYWORDS:**

Monoclonal antibody therapy; Zika virus; rhesus macaque; congenital disease; neutralizing antibody

3. **ACCOMPLISHMENTS:**

Table. 1 Major goals of the project as stated in the approved SOW (current reporting period: 1-6 months).

Specific Aim 1. To define mAb mutations that result in enhanced transplacental transport.				
Major Task 1	**Timeline** (months)	**Site 1**	**Site 2**	***Percentage of completion***
Design, synthesize, express antibody constructs; DNA sequencing of plasmids and viruses	**1-8**	**Dr. Magnani**		**40%**
Electroporate mAbs *in vivo*	6-12	Dr. Magnani	Dr. Rakasz	Not started
in vivo mAb level determination by ELISA	6-12	Dr. Magnani		Not started
Milestone(s) Achieved: Rank Ig constructs with highest transplacental transport rates	*12*	Dr. Magnani		Not started
Specific Aim 2. Can a fetus-targeted mAb prevent congenital ZIKV infection?				
Major Task 2	**Timeline** (months)	**Site 1**	**Site 2**	***Percentage of completion***
Produce recombinant monoclonal antibody	13-16	Dr. Magnani		Not started
Administer antibody to pregnant macaques, followed by ZIKV challenge	13-18	Dr. Magnani	Dr. Rakasz	Not started
in vivo mAb level determination by ELISA	13-18	Dr. Magnani		Not started
Zika viral loads	13-18	Dr. Magnani		Not started
Milestone(s) Achieved: Define efficacy of fetus-targeted mAb in preventing fetal ZIKV-replication	18	Dr. Magnani		Not started

Our initial experiments were set to design and characterize monoclonal antibody constructs to enhance the FcRn function (Table 1). During the current reporting period (months 1-6), our only major task as per our approved Scope of Work (SOW) was to: *Design, synthesize, express antibody constructs; DNA sequencing of plasmids and viruses.* Our major activities, specific objectives, and significant results were as follows:

- We have secured a challenge stock of Zika virus, a primary isolate from Rio de Janeiro (ZIKV U-1/16). This isolate is described in the publication below and was re-sequenced recently:

  Isolation of Infective Zika Virus from Urine and Saliva of Patients in Brazil.
  Bonaldo MC, Ribeiro IP, Lima NS, Dos Santos AA, Menezes LS, da Cruz SO, de Mello IS, Furtado ND, de Moura EE, Damasceno L, da Silva KA, de Castro MG, Gerber AL, de Almeida LG, Lourenço-de-Oliveira R, Vasconcelos AT, Brasil P. PLoS Negl Trop Dis. 2016 Jun 24;10(6):e0004816. doi: 10.1371/journal.pntd.0004816. eCollection 2016 Jun. PMID: 27341420

- We designed DNA vectors to express the ZIKV-neutralizing antibody SMZAb2 in wild-type and mutated versions to modify the interactions with FcRn.

- We will express our ZIKV-neutralizing antibodies as bi-specific molecules with mutations to enhance FcRn-binding. Therefore, we have identified and implemented a variety of the methods to characterize bi-specific antibody constructs and their affinity to FcRn *in vitro*:

Bispecific antibody characterization techniques

The controlled Fab arm exchange method of bispecific antibody generation utilizes complementary mutations in the CH3 domain of IgG1 to facilitate bi-specific molecule formation. When two antibodies containing these mutations are combined and the disulfide bonds connecting the Fab arms reduced, the Fab arms preferentially recombine with the heterologous arm at an efficiency of >90% (Figure 1).

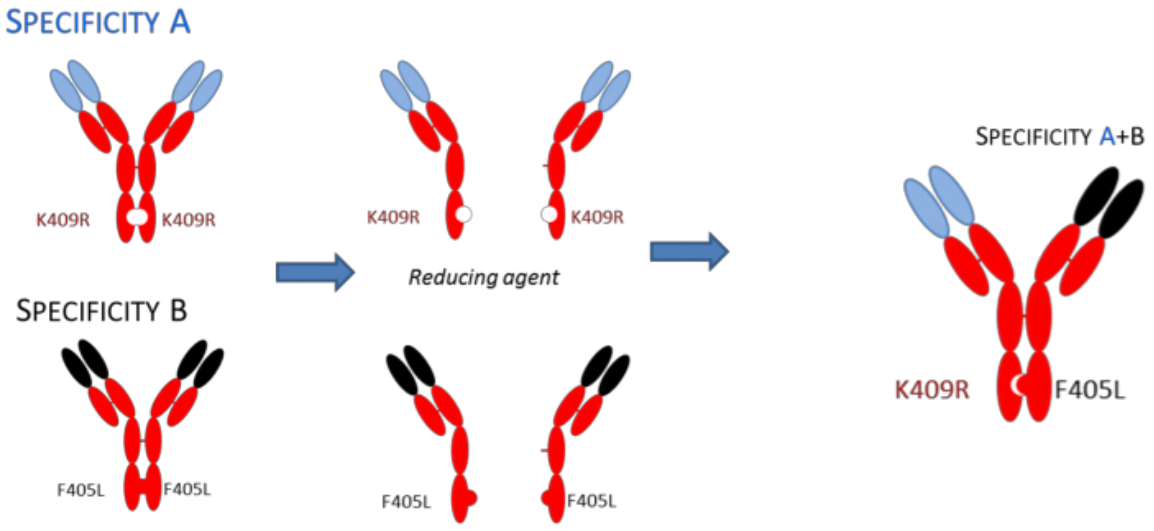


Figure 1. Schematic of bispecific antibody generation by controlled Fab arm exchange.

The bispecific antibody generation platform was developed in the Site 1 by the NIH nonhuman primate reagent resource (NHPRR) with assistance of the MassBiologics Process Development group. During that process, two antibodies with the K409R or F405L mutations are made and expressed using our standard antibody expression

platform. These antibodies are combined in equimolar amounts in the presence of the reducing agent (50 mM 2-mercaptoethylamine) to separate the antibody chains. The reducing agent is then removed by diafiltration against PBS, pH 7.4. The efficiency of bispecific antibody production is measured by hydrophobic interaction chromatography (Figure 2).

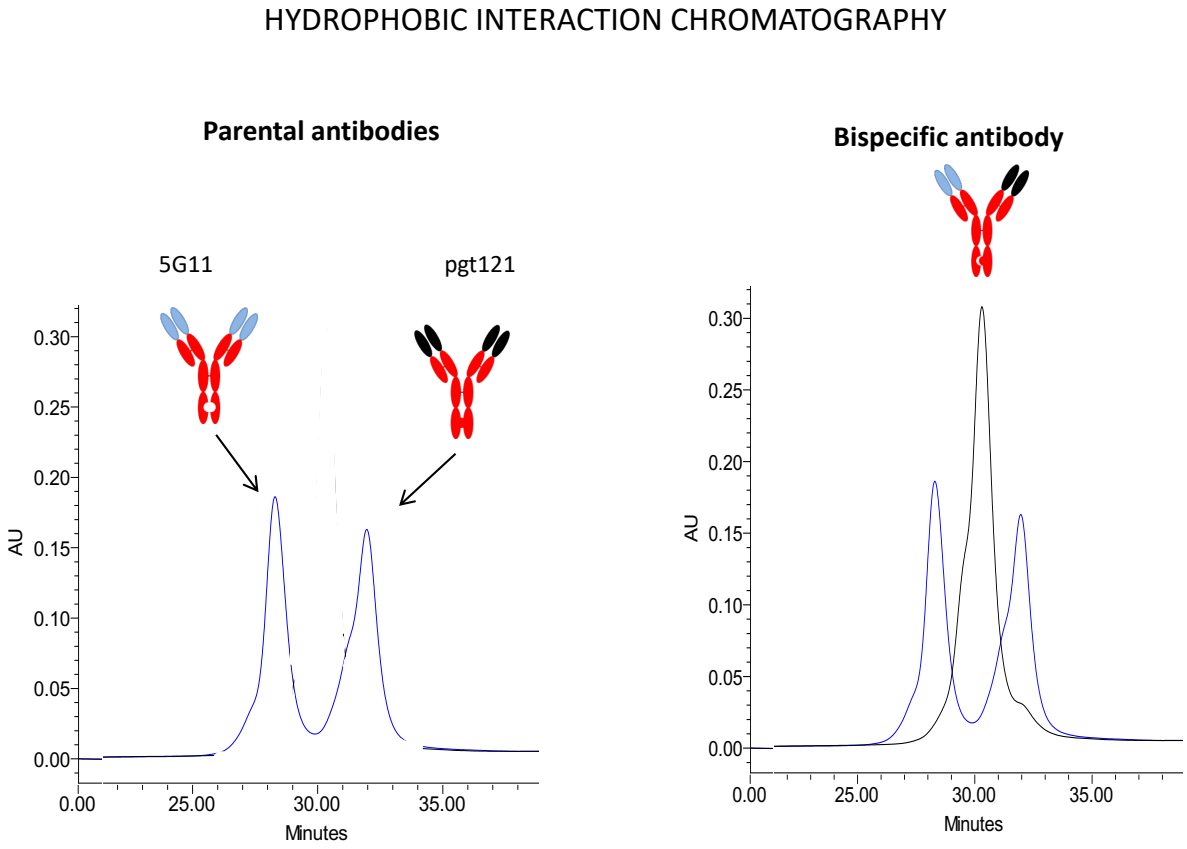


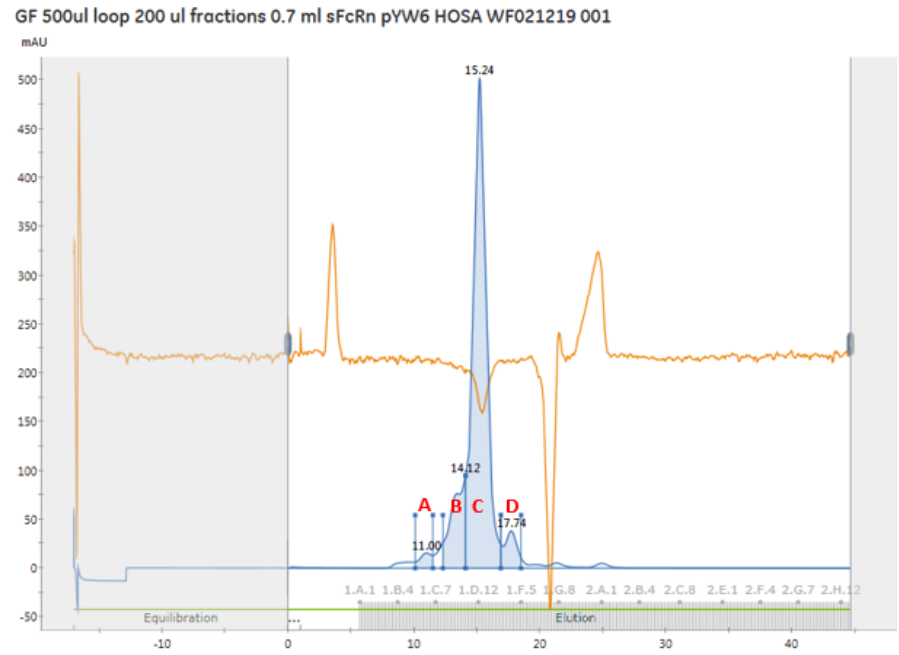
Figure 2. Characterization of the NHPRR bispecific antibodies by hydrophobic interaction chromatography can distinguish parental from bispecific antibodies. Plotting of hydrophobic interaction chromatography results for parental and bispecific antibodies demonstrates that the hydrophobicity characteristics of the bispecific antibody product lie mid-way between the parental antibodies. *We will now perform the same biochemical techniques for distinguishing parental and bispecific antibodies for the anti-ZIKV bi-specific constructs.*

In vitro FcRn-binding techniques

We have implemented methods to express soluble FcRn molecules in order to characterize the FcRn-mAb affinity of the bi-specific antibodies containing FcRn-enhancing mutations.

FcRn expression and purification

Truncated human FcRn were co-transfected into CHO cells with B2M for the production of soluble molecules. The transfected supernatant was purified with a nickel column and the product was formulated into phosphate-buffered saline solution (Figures 3, 4). The authenticity of the purified protein fractions was confirmed by ELISA assays using anti-his (Figure 5) and commercial anti-FcRn antibodies (Figure 6).



Peak Table - UV

Peak	Retention ml	Area mAU*mAU	Area %	Ext coeff. mg ml⁻¹ cm⁻¹	Fraction(s)	Volume ml	Amount mg	Concentration mg/ml	Conductivity mS/cm
Peak A	11.002	16.97	2.25	1.500	1.B.11 - 1.C.5	1.399	0.057	0.040	16.22
Peak B	14.118	111.7	14.81	1.500	1.C.10 - 1.D.6	1.800	0.372	0.207	16.21
Peak C	15.240	582.4	77.18	1.500	1.D.7 - 1.E.8	2.801	1.941	0.693	16.20
Peak D	17.739	43.49	5.76	1.500	1.E.9 - 1.F.4	1.600	0.145	0.091	16.22

Figure 3. Purification of soluble human FcRn by size exclusion chromatography (SEC). Fractions A, B, C, and D were collected for additional characterization.

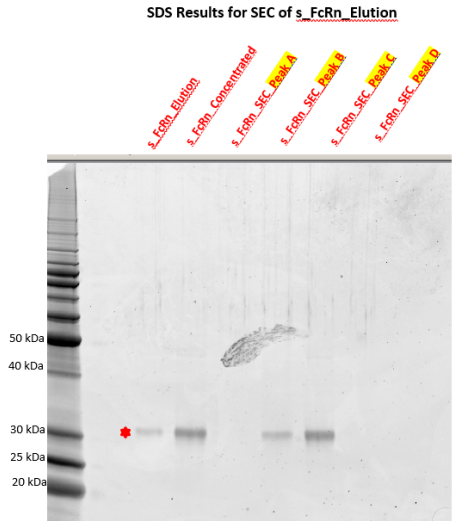


Figure 4. Electrophoresis of soluble FcRn post SEC elution. After running the elution through the SEC, the protein was loaded into an SDS for purity evaluation. The recombinant FcRn protein migrated on the electrophoresis gel at the expected size (32 kDa).

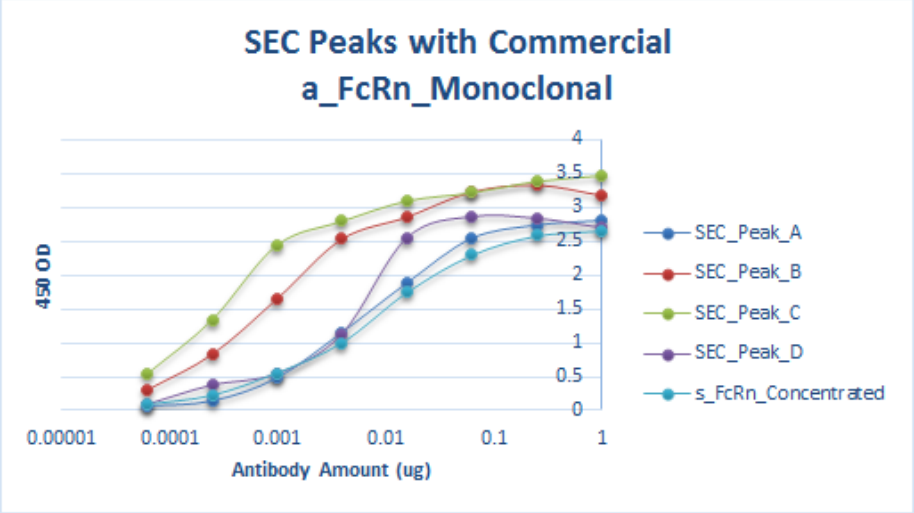


Figure 5. Anti-his ELISA of soluble FcRn SEC elutions to confirm the presence of the his-tagged recombinant FcRn.

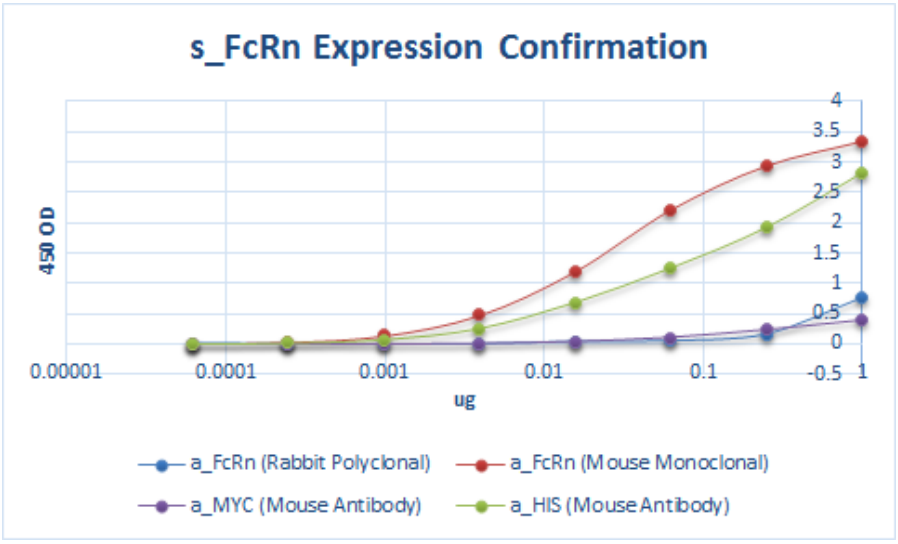


Figure 6. Anti-FcRn ELISA of soluble FcRn SEC elutions. Reactivity of the mouse anti-FcRn was used to authenticate the produced recombinant FcRn protein.

What opportunities for training and professional development has the project provided?

The PI (Diogo Magnani) attended the Keystone conference 'Antibody as Drugs' in 2019. He participated in the in the 'Engineering (Bispecific) Antibodies' workshop which helped in refining the critical assays needed to validate the bi-specific products that will be generated in this grant.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

The next reporting period (months 7-18) will contain the results of our most important milestones (Table 1). To accomplish our goals, we will express the constructs designed during this first reporting period and test them in the rhesus macaque model of ZIKV infection during pregnancy. We will produce the recombinant antibodies and finish the *in vitro* biochemical characterization in the next 3-6 months. We anticipate macaques in the second or third trimester of pregnancy becoming available in the breeding period of December-March. We will then perform the preclinical animal experiments to determine if an engineered ZIKV-neutralizing antibody with FcRn-enhancing mutations can cross the placental barrier at higher rates than wild type.

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. **CHANGES/PROBLEMS:**

Changes in approach and reasons for change

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them

The preclinical animal experiments proposed in this project depend largely on the availability of pregnant rhesus animals during the next 12 months. Recently, the Wisconsin National Primate Research Center experienced problems with supplying pregnant animals for all investigators. While this shortage has not impacted the current reporting period, it is possible that these animal supply issues will remain. Shall these problems extend into in the next granting period, the PI will request a modification of the animal contract site to the University of California Davis California National Primate Research Center (these plans were already discussed with both centers). Note that no work was done at the Wisconsin site to date.

Changes that had a significant impact on expenditures

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report.

Significant changes in use or care of human subjects Significant changes in use or care of vertebrate animals. Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. **PRODUCTS:**

Nothing to Report.

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

What individuals have worked on the project?

Name:	*Diogo Magnani*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-1773-2406*
Nearest person month worked:	1
Contribution to Project:	*Dr. Magnani oversees all steps of the project, including experimental planning, execution, analysis and reporting.*
Funding Support:	*No change.*

Name:	*Walter Flores*
Project Role:	*Research Associate*
Researcher Identifier (e.g. ORCID ID):	*Not available*
Nearest person month worked:	2
Contribution to Project:	*Mr. Flores is responsible for producing and qualifying the recombinant proteins used in this project. He also performs the in vitro assay analyses.*
Funding Support:	*No change.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

What other organizations were involved as partners?

Nothing to Report.

8. **SPECIAL REPORTING REQUIREMENTS**

COLLABORATIVE AWARDS:

Nothing to Report.

QUAD CHARTS:

Nothing to Report.

9. **APPENDICES:**

Nothing to Report.